

ECOLOGICAL ROLE OF CYTOTOXIC ALKALOIDS: *HALICLONA* N.SP., AN UNUSUAL SPONGE/ DINOFLAGELLATE ASSOCIATION

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Garson, M.J., Clark, R.J., Webb, R.I., Field, K.L., Charan, R.D. & McCaffrey, E.J. 1999 XX XX: Ecological role of cytotoxic alkaloids: *Haliclona* sp. nov., an unusual sponge/ dinoflagellate association. *Memoirs of the Queensland Museum* 44: 205-213. Brisbane. ISSN 0079-8835.

Light microscopy and electron microscopy studies of the tropical marine sponge *Haliclona* sp. nov. (Haplosclerida; Chalinidae) from Heron Island, Great Barrier Reef, have previously revealed the characteristic presence of a dinoflagellate symbiont and nematocysts. The dinoflagellates are morphologically similar to *Symbiodinium microadriaticum*, the common intracellular zooxanthellar symbiont of corals. The sponge grows on coral substrates, from which it may acquire the dinoflagellates and nematocysts. Chemical investigations found the sponge contained a suite of cytotoxic alkaloids, the haliclonacyclamines. Our investigations showed that these alkaloid metabolites cause significant coral tissue necrosis at concentrations of 5ppm after 160mins exposure in laboratory-based assays. At higher concentrations (10ppm and above) toxic effects were noted within 10mins exposure to the alkaloid fraction. Coral tissue necrosis was also observed after 40mins exposure to the major alkaloid component haliclonacyclamine A. In field experiments, the alkaloids were absorbed onto synthetic pads which were tied onto coral fingers. In both short term (10hrs) and long term (30hrs) experiments, coral tissue necrosis was observed at concentrations of 0.025% and above. We determined that a dose of 0.24% was equivalent to the natural exposure of coral pieces to sponge tissue, with our data indicating that haliclonacyclamines are effective toxins against coral tissue at lower than natural concentrations. When tested against natural populations of reef fish, the haliclonacyclamines were found to be potent feeding deterrents at ecologically-relevant concentrations (0.1% of sponge wet weight). □ *Porifera, Haliclona, Acropora, alkaloids, dinoflagellates, feeding deterrent, haliclonacyclamines, percoll density gradient fractionation, secondary metabolites, toxins, Symbiodinium microadriaticum.*

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Sponges are a major component of benthic fauna, representing the second largest biomass on tropical reefs after corals. The production of bioactive chemicals by marine sponges is a factor which likely enhances their competitiveness in coral reef environments. The secondary metabolites may act as chemical defenses against predation by fish, molluscs or other carnivores (reviewed in Paul, 1992), or act to prevent other marine species growing adjacent to or on top of the sponge tissue (Davis et al., 1989; Clare, 1996; Fusetani, 1997). Although sponge-derived chemicals have been implicated in allelochemical interactions with neighbouring corals (Sullivan et al., 1983; Porter and Targett, 1988),

few rigorous ecological studies have yet been undertaken.

Field studies by McCaffrey (1988) discovered a haplosclerid sponge, *Haliclona* sp. nov. (Haplosclerida: Chalinidae), which grows on coral substrates in the channel zones of Heron island at 10-14m depth. Although the sponge tissue was soft and easily torn, there were no feeding scars to indicate predation by fish or other scavengers, nor was its surface fouled by epiphytes; these facts suggested the presence of inhibitory chemicals. The sponge also exuded mucus upon collection. McCaffrey (1988) showed that the sponge contained antimicrobial components toxic to hydroids, corals, crustaceans and fishes, although she did not identify the

chemicals involved. Our subsequent research found crude organic extracts of this species exhibited potent antifungal and antimicrobial activity and an IC₅₀ of 5 µg/mL in a P388 mouse leukaemia assay. The aqueous methanol phase of a toluene:methanol (3:1) sponge extract was therefore extracted with chloroform, and the combined organic extracts processed to give a suite of novel alkaloids, the haliclonaclamines A-D (Fig. 1) (Charan et al., 1996; Clark et al., 1998). Using Percoll gradient centrifugation of fixed cells, we demonstrated that the alkaloids are stored in, and are therefore likely biosynthetic products of, sponge cells (Garson et al., 1998).

Haliclona sp. nov. has been reported to grow on coral substrate, usually *Acropora nobilis*, but also other corals such as *Poecilopora* sp. and *Seriatopora hytrix* and also on sand-covered coral rock (McCaffrey, 1988). When the sponge tissue was examined by light microscopy, nematocysts of mean length 12–15 µm were detected, as was a dinoflagellate which morphologically resembled *Symbiodinium microadriaticum*, the dinoflagellate symbiont of reef corals (McCaffrey 1988, Garson et al., 1998).

Haliclona sp. nov. is a versatile sponge in that it appears to have evolved multiple defense strategies. In addition to the potential physical defense provided by mucus exudation, and the presence of nematocysts, the associated alkaloids may provide an additional chemical defence *in situ*. In this paper, we present some preliminary evidence on the ecological roles of the haliclonaclamine alkaloids.

MATERIALS AND METHODS

CHEMICALS AND BIOCHEMICALS. Agar was purchased from Sigma Chemical Company

(MO, USA) while brine shrimp eggs and dried krill were purchased from an aquarium supply shop. Solvents used in ecological experiments and in the extraction of compounds from whole tissue or cell separation experiments were glass distilled.

BIOLOGICAL MATERIALS. Samples of *Haliclona* sp. nov. were collected by hand using SCUBA at the Coral Gardens (10–15m depth), Heron Island (23°27'S, 151°55'E), S Great Barrier Reef, Australia, under permit numbers G96/050, G97/097, G98/037 issued jointly by the Great Barrier Reef Marine Park Authority (GBRMPA) and the Queensland National Parks and Wildlife Service; and at North Point, Lizard Island (14°39'S, 145°27'E), N Great Barrier Reef under GBRMPA permit G98/227. Sponge samples used in biological experiments were maintained in running sea water at ambient temperature and light conditions prior to use. Coral samples used for ecological studies were collected under GBRMPA permits G97/097 and G98/037. For a brief description of the sponge and the dinoflagellate symbiont, see Charan et al. (1996) and Garson et al. (1998). A voucher specimen of the sponge is accessioned in the Queensland Museum, Brisbane, collections (QM G304086).

ISOLATION OF METABOLITES. A crude alkaloid extract (600mg) was prepared from frozen sponge (250g wet wt.) as described by Clark et al. (1998) and further purified by normal phase HPLC using EtOAc/hexanes/Et₃N (30:65:5 or 80:15:5) to give haliclonaclamines A (Fig. 1A; 162mg, 0.065%), B (Fig. 1B; 144mg, 0.057%), C (Fig. 1C; 26mg, 0.0012%) and D (Fig. 1D; 5mg, 0.002%).

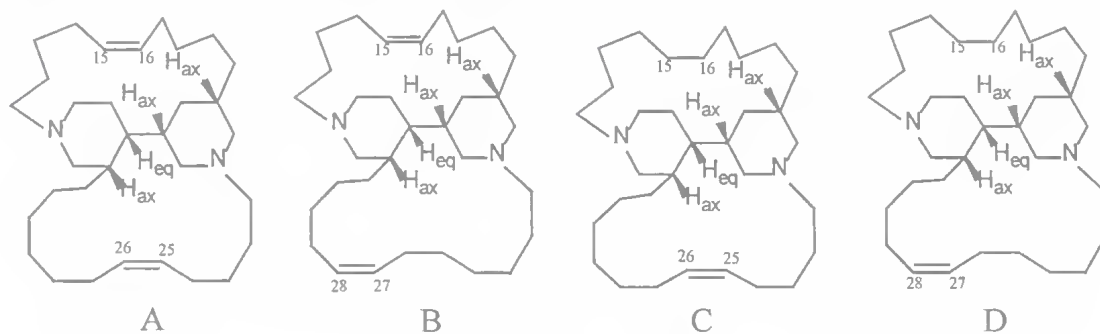


FIG. 1. Structures and stereochemistry of the haliclonaclamines. A, Haliclonaclamine A. B, Haliclonaclamine B. C, Haliclonaclamine C. D, Haliclonaclamine D.

ESTIMATIONS OF NATURAL CONCENTRATIONS. To determine the average natural concentration of metabolite in the sponge, three different samples of *Haliclona* sp. nov. collected from the Coral Gardens dive site were extracted, and the alkaloid content estimated. The ratio of the different haliclonyclamines in each extract was assessed by normal phase HPLC using EtOAc/hexanes/Et₃N (30:65:5). For comparison, a specimen collected at Coral Spawning dive site, 500m further along the reef, was extracted. For toxicity trials, a thin slice of frozen sponge (10mm x 20mm x 1mm; approx 1g wet wt.) was extracted; this piece of sponge was equivalent in volume to a pad used for the *in situ* coral toxicity trials. The exudation rate of alkaloids from the sponge was assessed by aerating a 33g piece of sponge in seawater for 3hrs 45mins in ambient temperature and light under flow conditions. The sponge was carefully removed, and the residual sea water filtered through a 0.22µm filter, then passed through a C₁₈ Seppak cartridge, which was flushed with 100ml DCM to flush out organic components. Removal of the DCM solvent left a residue (3.9mg) which was analysed by TLC and NMR.

MICROSCOPY STUDIES. Tissue samples were processed as described previously (Garson et al., 1998). Sections were viewed using Hitachi H-800 and Jeol 1010 transmission microscopes. Light microscopic observations of tissue or cell preparations were made on an Olympus BH-2 microscope using Nomarski interference optics.

FISH FEEDING DETERRENCY STUDIES. Agar cubes were prepared by combining 30g of agar, 2.7g of brine shrimp eggs and 2.7g of krill in one litre of Milli-Q water. This mixture was heated to 85°C and allowed to cool to approximately 50°C when the alkaloids were added to the agar mixture at 0.1% wt/vol (half the estimated natural concentration). The mixture was then cooled to approximately 40°C before being poured into ice cube trays. Each cube contained a 1cm² piece of wire gauze to which a length of dental floss was attached. Seven cubes of either treatment or control were then attached to a polypropylene rope by the dental floss with a 25cm gap between each cube. Eleven sets of paired ropes (one control and one treatment rope) were placed at the Coral Gardens field site at a depth of approximately 14m and with no more than 0.5m between the ropes. Divers stayed in the water to monitor feeding. When approximately half the cubes were eaten (approx 1hr), the ropes

were collected and the number of cubes eaten counted. Data was analysed with Wilcoxon's signed rank test (Zar, 1984); two-tailed *p*-values are reported. The haliclonyclamines remained present in the agar cubes throughout the assay (TLC confirmation at the end of the experiment).

CORAL TOXICITY STUDIES. *Laboratory experiments.* Pieces of *Acropora* sp. were collected and placed in an aquarium with continuous water flow for a period of 12hrs under ambient conditions of temperature and light. Pieces (approximately 2cm long) were broken off carefully and left in the aquarium for a further 10hrs to acclimatise. Treatments were prepared by dissolving the crude alkaloid extract in ethanol (at a concentration of 6mg/mL), then aliquots were dispensed into voucher jars containing 100mL of filtered sea water to give final concentrations of 40ppm, 10ppm, and 5ppm. A fourth treatment was prepared consisting of haliclonyclamine A at 10ppm. A control experiment contained 666µL EtOH. There were ten replicates of each treatment and of the control. The solutions were aerated throughout the duration of the experiment. A single piece of coral was placed in each voucher jar and observed after 10mins, 20mins, 40mins, 80mins, 160mins and finally after an interval of 9.5hrs. The condition of the coral pieces was graded according to the following five point scale (Aceret et al, 1995): 1=75-100% of colony exhibiting normal polypal activity, with extended tentacles, no change in pigmentation, no mortality; 2=50-75% as above; 3=less than 50% as above; 4=tissue still evident, obvious loss of pigmentation, decreased water clarity, and no visible signs of life; 5=little or no remaining tissue evident, complete loss of pigmentation, mortality.

Field experiments. Absorbent pads (1x2cm; thickness 0.1cm) were impregnated with alkaloid at concentrations of 0.005, 0.01, 0.025, 0.05, 0.1 and 0.4% (10 replicates at each concentration, dissolved in 200µL DCM per pad). The control consisted of a pad impregnated with 200µL of DCM. The pads were taken underwater in plastic bags and attached to coral fingers with cable ties. The pads were left for 24hrs after which the coral was carefully detached using small pliers, taken back to the lab and left in the aquarium for 6hrs to acclimatise. The pads were removed and the condition of the coral graded (using the same scale as for the lab experiments). In a second

shorter term experiment using 0.01, 0.025, 0.035, 0.05 and 0.075% alkaloid, the pads were left underwater for 8hrs, then acclimatised in aquaria for 2hrs prior to grading. At the end of each experiment, the pads were extracted with dichloromethane to confirm the presence of residual alkaloids. In the 10hr experiment there were residual alkaloids present at all concentrations tested while in the 30hr experiment only the 0.4% treatment still contained residual alkaloid.

RESULTS

CHEMISTRY. The structures and stereochemistry of the haliclonaacyclamine metabolites A-D are shown in Figure 1A-D. The metabolites were characterised by 2D-NMR spectroscopy and by single crystal x-ray analysis (Charan et al., 1996, Clark et al., 1998). Haliclonaacyclamines A and B (Fig. 1A-B) have $\delta^{25,26}$ or $\delta^{27,28}$ double bonds respectively in addition to a $\delta^{15,16}$ double bond, while haliclonaacyclamines C and D (Fig. 1C-D) were found to be analogous to haliclonaacyclamines A and B respectively, but lacking the $\delta^{15,16}$ double bond. The stereochemistry of the $\delta^{15,16}$, $\delta^{25,26}$ or $\delta^{27,28}$ double bonds was found to be Z in all metabolites (Charan et al., 1996, Clark et al., 1998).

ESTIMATION OF NATURAL CONCENTRATIONS. The average yield of alkaloid crude extract from the three Coral Gardens samples of *Haliclona* sp. nov. was $0.25\% \pm 0.01\%$ of sponge wet weight; the ratio of the haliclonaacyclamines estimated by HPLC was found to be consistent between all three extracts. The yields of individual alkaloids were: 1A, 0.065%; 1B, 0.057%; 1C, 0.012%; and 1D, 0.002%, giving a combined isolation yield of 0.136%. Some losses of compound are expected to occur during purification. The alkaloid yield from the specimen collected at Coral Spawning dive site was $0.24\% \pm 0.01\%$ of sponge wet weight; the composition of haliclonaacyclamines was similar to that at Coral Gardens by HPLC. The 1g piece of sponge of equivalent volume to a single pad used in the *in situ* trials contained 2.4mg alkaloid. The rate of leaching of chemicals from *Haliclona* sp. nov. was assessed in laboratory experiments under flow conditions. An organic extract was obtained from sea water in which the sponge was immersed; TLC and ^1H NMR analysis detected the haliclonaacyclamines in this extract. The amount of alkaloid detected using these analytical techniques is estimated to be 1.58mg.

Therefore if the haliclonaacyclamines were present in the surrounding water, as suggested by McCaffrey (1988), then the rate of exudation was estimated to be 0.013mg/hr/g of sponge.

BIOLOGY. *Haliclona* sp. nov. is one of the dominant sponges of the channel zone at Heron Island, where it commonly occurs at depths of 10-15m at the base of the reef slope. The sponge grows on coral substrate, and when damaged or collected, exudes abundant mucus. Its preferred substrate is *Acropora nobilis*, however specimens have also been observed to grow on *Styloporapistillata*, *Pocillopora* sp., *Seriatopora lytrix* and on sand-covered coral rock (McCaffrey, 1988). Usually the sponge is a uniform olive-brown colour, but infrequently the tips are bleached. By light and transmission electron microscopy, dinoflagellates, usually intracellular, and nematocysts were present throughout preparations from sponge samples collected growing on acroporid substrates (Garson et al., 1998). Infrequently samples contained low populations of nematocysts, for example when collected from sand-covered coral rock.

In June 1998, after an El Niño period, a bleached specimen of *Haliclona* sp. nov. was found growing on bleached acroporid coral at Heron Island. By microscopy, the sample was free of nematocysts, but contained some dinoflagellates; these were not healthy in appearance and were free-living rather than intracellular. Samples of the sponge were also found on dead coral substrate at North Point, Lizard Island; these specimens were small in size and always had bleached tips relative to the brown body colour. By microscopy, the bleached tips were free of both dinoflagellates and nematocysts; in contrast, the body of the sponge, which was a brown colour, contained healthy dinoflagellates but had no nematocysts.

ECOLOGY. *Fish deterrency.* The crude alkaloid fraction of *Haliclona* sp. nov. deterred feeding (mean deterrency = 4.6 ± 0.6 of 7 cubes eaten; $N=11$; $p=0.002$) by natural populations of reef fish in field assays conducted at 14m depth in the channel at Heron Island (Fig. 2). The alkaloid fraction was tested at half average natural concentration (0.1% of wet weight; 16mg per agar cube). Rabbitfish (*Siganus argenteus*) were a major consumer of cubes in this experiment.

Toxicity towards scleractinian corals. Figure 3 shows the results of laboratory experiments in which an alkaloid fraction from *Haliclona* sp.

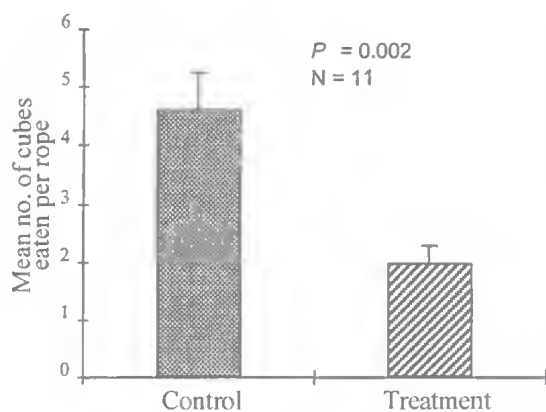


FIG. 2. Field assays of a crude alkaloid extract (tested at half natural concentration, 0.1% of wet wt.) from *Haliclona* sp. nov. at Heron Island. *P*-values calculated using a Wilcoxon two-tailed sample test.

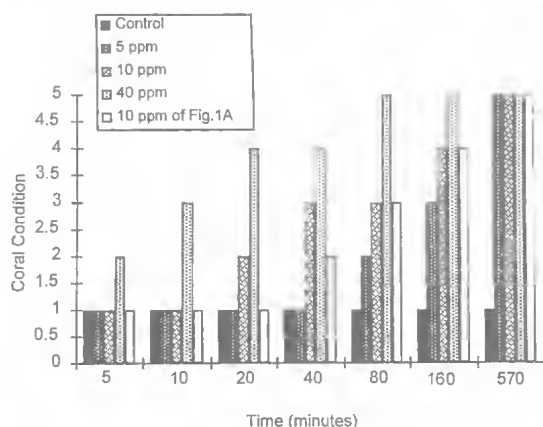


FIG. 3. Toxicity of an alkaloid fraction from *Haliclona* sp. nov. towards tips of *Acropora* sp. in laboratory experiments. Alkaloid concentrations ranged from 5-40ppm. Number of corals per concentration=10.

nov. was added at varying concentrations to small aquaria of filtered seawater containing tips of *Acropora* sp. At 5ppm concentration, less than 50% of the corals were fully viable, that is exhibiting normal polypal activity, with tentacles extended and no loss of pigmentation after 160mins. At higher concentrations (10ppm and above), toxic effects were noted within 10mins of exposure to the alkaloid fraction. At the highest concentration tested (40ppm), all the corals were killed within 80mins. The major alkaloid component haliclonyclamine A (Fig.1A) was tested at a single concentration of 10ppm. The toxic

effect of haliclonyclamine A (Fig. 1A) was not as rapid as the alkaloid mixture was at 10ppm, but was equally effective in inhibiting coral and polypal activity after 80mins. Significant coral tissue necrosis was detected after 40mins exposure to this metabolite. In control experiments, dichloromethane solvent alone was added to the aquarium water without adverse effect to the coral pieces.

Field assays were carried out using a method based on the work of Porter & Targett (1988). Alkaloid extracts were coated onto synthetic sponge pads and tied to healthy coral pieces growing in the vicinity of *Haliclona* sp. nov. in the channel at Heron Island. Control pads (coated with dichloromethane solvent only) produced no effects, whereas pads containing 0.025% alkaloid resulted in less than 50% of the corals tested remaining viable after 30hrs (Fig. 4). In a shorter term experiment (10hrs duration; Fig. 5), the corals became unviable at concentrations of 0.025% alkaloid.

DISCUSSION

In her PhD work, McCaffrey (1988) demonstrated feeding deterrence of crude extracts from *Haliclona* sp. nov. by the bream, *Acanthopagrus australis*. Our field experiments have now demonstrated the deterrence of the haliclonyclamine alkaloids to reef fish at ecologically-relevant concentrations. There is an increasing body of experimental evidence which demonstrates the deterrence of sponge secondary metabolites to fish predators (Rogers & Paul, 1991; Paul, 1992; Pennings et al., 1994; Pawlik et al., 1995; Chanas et al., 1996; Uriz et al., 1996). There is no obvious correlation between metabolite type and feeding deterrence since the range of structures which have been identified as feeding deterrents includes alkaloids (Chanas et al., 1996), sesterterpenes (Rogers & Paul, 1991; Duffy & Paul, 1992; Pennings et al. 1994), sesquiterpenes (Pennings et al., 1994; Uriz et al., 1996), and brominated metabolites (Paul, 1992; Pennings et al. 1994; Chanas et al., 1996). A number of these studies have considered other factors which may impact on palatability such as nutritional quality (Duffy & Paul, 1992; Chanas & Pawlik, 1995), the presence of spicules or the texture of the sponge tissue (Chanas & Pawlik, 1995; Chanas & Pawlik, 1996; Uriz et al., 1996). Some chemically-defended sponges contain spicules (Uriz et al., 1992; Chanas & Pawlik, 1995; Uriz et al., 1996). We have not yet investigated whether the spicules present in

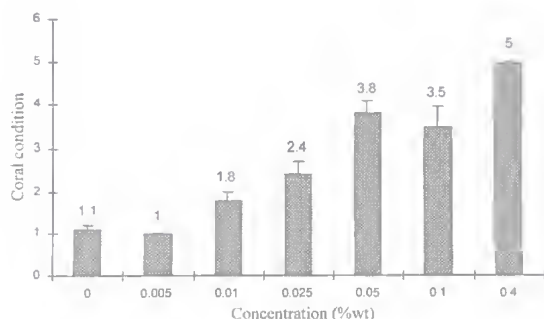


FIG. 4. Toxicity of an alkaloid fraction from *Haliclona* sp. nov. towards the periphery of *Acropora* sp. in field experiments. Alkaloid concentrations ranged from 0.005-0.4%. Number of corals per concentration=10. Length of experiment 30hrs. See text for coral toxicity gradation scale.

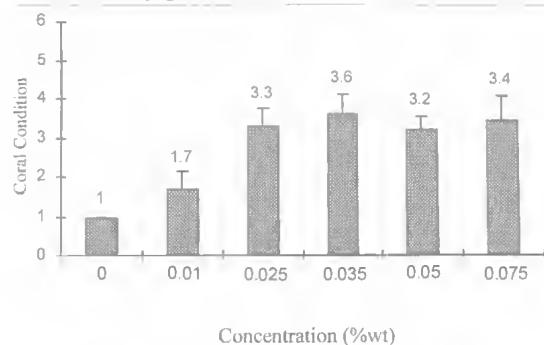


FIG. 5. Toxicity of an alkaloid fraction from *Haliclona* sp. nov. towards the periphery of *Acropora* sp. in field experiments. Alkaloid concentrations ranged from 0.01-0.075%. Number of corals per concentration=10. Length of experiment 10hrs. See text for coral toxicity gradation scale.

Haliclona sp. nov. are an additional deterrent to fish, or whether they simply play a structural role in this fragile sponge - although it is speculated that the former is unlikely given that spicules are small, smooth, homogeneous oxas contained completely within the choanosome (J. Hooper, pers. comm.). Further experiments are also required to assess the effect of nutritional quality on the anti-feedant properties of the haliclona-cyclamines.

Marine sponges are known to release metabolites directly into the water column (Walker et al., 1985) or indirectly through a mucus exudate (Sullivan et al., 1983). McCaffrey (1988) investigated the exudation of biologically-active compounds from *Haliclona* sp. nov., but did not identify the chemicals or measure the exudation

rate. In preliminary laboratory experiments we have estimated the exudation of *Haliclona* metabolites is 0.013mg/hr/g wet weight of sponge under flow conditions. Our estimates took no account of the effect of water throughput or current or the concentration of alkaloids in the mucus exudate. Measurement of the natural leaching rate of organic extracts from marine sponges has not yet been addressed in the literature, although Henrikson et al. (1995) have measured the release of sponge chemicals from a range of artificial substrates. A detailed quantitative study of alkaloid leaching from *Haliclona* sp. nov. is in progress in our laboratory.

In laboratory experiments, a mixture of the haliclona-cyclamine alkaloids exhibited toxic effects towards pieces of acroporid coral and caused the corals to release mucus and to shed symbiotic zooxanthellae. When a sample of haliclona-cyclamine A was tested at 10ppm, coral tissue necrosis was observed, however the purified metabolite was less toxic than the alkaloid mixture tested at the same concentration during short term exposure. These data suggest that the alkaloid mixture may be a more effective toxin than the individual chemicals. Further experiments will be required to confirm this synergistic effect.

Our field results also confirmed the effective toxicity of the alkaloids. These experiments showed that the metabolites in *Haliclona* sp. nov. actively inhibit the metabolism and tissue survival of adjacent acroporid corals. Our experiments used a range of alkaloid concentrations up to 0.075% (10hr experiment) or 0.4% (30hr experiment). If it is assumed that the alkaloids leach out of the artificial pads at the same rate as from sponge tissue, our experiment suggests that the metabolites are effective toxins at lower than natural concentrations. The field results cannot easily be related to the laboratory toxicity trials. The metabolite concentrations used in the coral pad experiment were equivalent to 250-20,000ppm, however the effective metabolite concentration that the coral may experience is much lower.

Since both direct contact (using synthetic sponge pads to mimic the effects of sponge tissue), and indirect contact (addition of metabolites to aquarium water), resulted in toxic effects on corals, we conclude that the *Haliclona* alkaloids are effective allelochemicals which enable the sponge to compete successfully for space with coral substrates (Jackson & Buss,

1975; Wulff & Buss, 1979; Porter & Targett, 1988). *Haliclona* sp. nov. is an aggressive sponge which may preferentially select coral substrates as habitat. Although some studies have demonstrated the effectiveness of inhibitory substances in improving the competitiveness of sponges for space among benthic organisms (Becerro et al., 1997; McCaffrey & Endean, 1985; Thompson, 1985; Thompson et al., 1985; Walker et al., 1985; Clare, 1996; Wright et al., 1997), other studies have shown a contrasting ecological effect, for example that the presence of sponge chemicals may induce marine invertebrate larvae to settle (Bingham & Young, 1991). The ecological effectiveness of *Haliclona* metabolites on benthic invertebrates other than corals, or on their larvae, is yet to be tested in our laboratory. This study will enable us to determine if the haliclonacyclamines are selectively toxic or not.

The *Haliclona* metabolites possess a lipophilic carbon backbone together with a polar amine functionality, and are therefore amphiphilic in character. The compounds, thus, have partial water solubility; for example, NMR spectra can be obtained in deuteriated water. The sponge pads placed underwater for 10hrs still retained alkaloids at the end of the experiment. In the long term exposure study, we observed loss of metabolites from the artificial pads at the lower concentrations. The ongoing release of a polar, diffusible substance by a sponge is of no value as a mechanism to inhibit settlement (Becerro et al., 1997), and is also metabolically uneconomic; the most suitable chemical candidates for defense or for use as an anti-fouling agent are likely to be water-insoluble. Some recent studies have attempted to better simulate natural conditions in field trials by embedding sponge extracts onto artificial matrices which can be placed in the field for long periods. Chemicals may leach out of these gel matrix at rates which may mimic their natural release (Morse et al., 1994; Hendrikson & Pawlik, 1995). Experiments of this type are currently in progress in our laboratory.

In corals, photosynthesis is performed uniquely by dinoflagellate (zooxanthellae) symbionts which supply the host with nutrients by translocation (Muscatine & Cernichiaro, 1969). Cyanobacteria are the most common sponge photosynthetic symbionts, however some groups of sponges, notably the boring sponges of the order Hadromerida (e.g. *Cliona* spp.), have been shown to contain zooxanthellae (Vacelet, 1982; Rützler 1990), although it is not yet known whether the dinoflagellate partners supply the

hadromerid sponges with photosynthetic products. We propose that *Haliclona* sp. nov. may poison or kill the coral tissue on which it grows in order to acquire dinoflagellate symbionts, which provide additional metabolic benefits to the sponge, thereby enhancing its competitiveness. Sullivan et al. (1983) showed that the boring sponge *Siphonodictyon* sp. (=Aka, family Phloeodictyidae), uses toxin-containing mucus to kill surrounding tissue. *Haliclona* sp. nov. exudes abundant mucus on collection and so may perhaps use a similar process. We are currently investigating the chemistry of *Haliclona* sp. nov. mucus to determine if it contains the haliclonacyclamines.

To our knowledge, no other marine sponge has been reported to contain nematocysts. Perhaps the nematocyst capture represents an additional serendipitous defense mechanism in *Haliclona* sp. nov. The high numbers of nematocysts found intracellularly in healthy sponge tissue samples are inconsistent with their casual acquisition from the surrounding water. Both nematocysts and dinoflagellates were absent in a bleached sample of *Haliclona* sp. nov. found growing on bleached coral at Heron Island. This sample was clearly stressed since the dinoflagellates appeared unhealthy and were free-living rather than intracellular. Partially-bleached samples of *Haliclona* sp. nov. collected from dead coral rock at Lizard Island were also free of nematocysts even if the body of the tissue was healthy and contained dinoflagellates. The distribution and specific source of the nematocysts present within the sponge tissue is currently under further investigation, as is their impact on predation.

ACKNOWLEDGEMENTS

We thank the director and staff of Heron Island Research Station, and undergraduate students from the coral reef ecology subject (ZL329) at The University of Queensland for field assistance. Dr John Hooper of the Queensland Museum identified the sponge sample for us. Our research on *Haliclona* sp. nov. is funded by a University of Queensland Special Program Grant and by the Australian Research Council.

LITERATURE CITED

- ACERET, T.L., SAMMARCO, P.W. & COLL, J.C. 1995. Toxic effects of alcyonacean diterpenes on scleractinian corals. *Journal of Experimental Marine Biology and Ecology* 188: 63-78.
BECERRO, M.A., URIZ, M.J. & TURON, X. 1997. Chemically-mediated interactions in benthic

- organisms: the chemical ecology of *Crambe crambe* (Porifera, Poccilosclerida). *Hydrobiologia* 356: 77-89.
- BINGHAM, B.L. & YOUNG, C.M. 1991. Influence of sponges on invertebrate recruitment: a field test of allelopathy. *Marine Biology* 109: 19-26.
- CHANAS, B. & PAWLIK, J.R. 1995. Defenses of Caribbean sponges against predatory reef fish. II. Spicules, tissue toughness and nutritional quality. *Marine Ecology Progress Series* 127: 195-211.
1996. Does the skeleton of a sponge provide a defense against predatory reef fish? *Oecologia* 107: 225-231.
- CHANAS, B., PAWLIK, J.R., LINDEL, T. & FENICAL, W. 1996. Chemical defense of the Caribbean sponge *Agelas clathrodes* (Schmidt). *Journal of Experimental Marine Biology and Ecology* 208: 185-196.
- CHARAN, R.D., GARSON, M.J., BRERETON, I.M., WILLIS, A.C. & HOOPER, J.N.A. 1996. Haliclonacyclamines A and B, cytotoxic alkaloids from the tropical marine sponge *Haliclona* sp. *Tetrahedron* 52: 9111-9120.
- CLARE, A.S. 1996. Marine natural product antifoulants: status and potential. *Biofouling* 9: 211-229.
- CLARK, R.J., FIELD, K.L., CHARAN, R.D., GARSON, M.J., BRERETON, I.M. & WILLIS, A.C. 1998. The haliclonacyclamines, cytotoxic alkaloids from the tropical marine sponge *Haliclona* sp. *Tetrahedron* 54: 8811-8826.
- DAVIS, A.R., TARGETT, N.M., MCCONNELL, O.J. & YOUNG, C.M. 1989. Epibiosis of marine algae and benthic invertebrates: Natural products chemistry and other mechanisms inhibiting settlement and overgrowth. Pp. 85-113 In Scheuer, P.J. (ed.) *Bioorganic marine chemistry*. Vol. 3. (Springer-Verlag: Berlin).
- DUFFY, J.E. & PAUL, V.J. 1992. Prey nutritional quality and the effectiveness of chemical defenses against tropical reef fishes. *Oecologia* 90: 333-339.
- FUSETANI, N. 1997. Marine natural products influencing larval settlement and metamorphosis of benthic invertebrates. *Current Organic Chemistry* 1: 127-152.
- GARSON, M.J., FLOWERS, A.E., WEBB, R.I., CHARAN, R.D. & McCAFFREY, E.J. 1998. A sponge dinoflagellate association in the haplosclerid sponge *Haliclona* sp.: cellular origin of cytotoxic alkaloids by Percoll density gradient fractionation. *Cell Tissue Research* 293: 365-373.
- IIENDRIKSON, A.A. & PAWLIK, J.R. 1995. A new antifouling assay method: results from field experiments using extracts of four marine organisms. *Journal of Experimental Marine Biology and Ecology* 194: 157-165.
- JACKSON, J.B.C. & BUSS, L.W. 1975. Allelopathy and spatial competition among coral reef invertebrates. *Proceedings of the National Academy of Science* 72: 5160-5163.
- McCAFFREY, E.J. 1988. Biologically-active compounds from marine sponges collected from Queensland waters. PhD thesis (University of Queensland: Brisbane).
- McCAFFREY, E.J. & ENDEAN, R. 1985. Antimicrobial activity of tropical and subtropical sponges. *Marine Biology* 89: 1-8.
- MORSE, D.E., MORSE, A.N.C., RAIMONDI, P.T. & HOOKER, N. 1994. Morphogen-based chemical flypaper for *Agaricia humilis* coral larvae. *Biological Bulletin* 186: 172-181.
- MUSCATINE, L. & CERNICHIARI, E. 1969. Assimilation of photosynthetic products of zooxanthellae by a reef coral. *Biological Bulletin* 137: 506-523.
- PAUL, V.J. 1992. Chemical defenses of benthic marine invertebrates. Pp. 164-168. In Paul, V.J. (ed.) *Ecological role of marine natural products*. (Comstock Publishing Associates: London).
- PAWLIK, J.R., CHANAS, B., TOONEN, R.J. & FENICAL, W. 1995. Defenses of Caribbean sponges against predatory reef fish. I. Chemical deterrence. *Marine Ecology Progress Series* 127: 183-194.
- PENNING, S.C., PABLO, S.R., PAUL, V.J. & DUFFY, J.E. 1994. Effects of sponge secondary metabolites in different diets on feeding by three groups of consumers. *Journal of Experimental Marine Biology and Ecology* 180: 137-149.
- PORTER, J.W. & TARGETT, N.M. 1988. Allelochemical interactions between sponges and corals. *Biological Bulletin* 175: 230-239.
- ROGERS, S.D. & PAUL, V.J. 1991. Chemical defenses of three *Glossodoris* nudibranchs and their dietary *Hyrtios* sponges. *Marine Ecology Progress Series* 77: 221-232.
- RÜTZLER, K. 1990. Associations between Caribbean sponges and photosynthetic organisms. Pp. 455-471. In Rützler, K. (ed.) *New perspectives in sponge biology* (Smithsonian Institution Press: Washington DC).
- SULLIVAN, B., FAULKNER, D.J. & WEBB, L. 1983. Siphonodictidine, a metabolite of the boring sponge *Siphonodictyon* sp. that inhibits coral growth. *Science* 221: 1175-1176.
- THOMPSON, J.E. 1985. Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. I. Biological evidence. *Marine Biology* 88: 23-26.
- THOMPSON, J.E., WALKER, R.P. & FAULKNER, D.J. 1985. Screenings and bioassays for biologically-active substances from forty marine sponge species from San Diego, California, USA. *Marine Biology* 88: 11-21.
- URIZ, M.J., MARTIN, D. & ROSELL, D. 1992. Relationships of biological and taxonomic characteristics to chemically mediated bioactivity in Mediterranean littoral sponges. *Marine Biology* 113: 287-297.
- URIZ, M.J., TURON, X., BECERRO, M.A. & GALERA, J. 1996. Feeding deterrence in sponges.

- The role of toxicity, physical defenses, energetic contents and life-history stage. *Journal of Experimental Marine Biology and Ecology* 205: 187-204.
- VACELET, J. 1982. Algal-sponge symbioses in the coral reefs of New Caledonia: a morphological study. *Proceedings of the 4th International Coral Reef Symposium* 2: 713-719.
- WALKER, R.P., THOMPSON, J.E. & FAULKNER, D.J. 1985. Exudation of biologically-active metabolites in the sponge *Aplysina fistularis*. II. Chemical evidence. *Marine Biology* 88: 27-32.
- WRIGHT, J.T., BENKENDORFF, K. & DAVIS, A.R. 1997. Habitat-associated differences in temperate sponge assemblages: the importance of chemical defence. *Journal of Experimental Marine Biology and Ecology* 213: 199-213.
- WULFF, J.L. & BUSS, L.W. 1979. Do sponges help hold corals reefs together? *Nature* 281: 474-475.
- ZAR, J.H. 1984. *Biostatistical analysis*. 2nd Edition. (Prentice-Hall: Englewood Cliffs, NJ).